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Isolation and Characterisation of Sulphur Oxidizing Bacteria Isolated from Hot Spring in Malaysia for Biological Deodorisation of Hydrogen Sulphide in Chicken Manure

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ABSTRACT

In this study, the isolation of sulphur oxidising bacteria (SOB) from hot spring in Malaysia was carried out in an enrichment culture using sodium thiosulphate as a sole energy and CO_2 as a sole carbon source. A total number of 80 SOB isolates were obtained from the agar plate and considered as positive SOB due to their abilities using thiosulphate for growth. All the isolates were initially screened for their fast growths in liquid medium and 13 isolates were selected for another screening process. Three SOB isolates namely isolate AH18, AH25, and AH28 were selected based on their abilities to grow faster, produce the highest sulphate ion and reducing the pH in the growth medium. The cells were Gram-negative and short rod-shaped. The effects of various variables including temperature (25-45 °C), pH (4-9), sodium thiosulphate concentrations (4-100 mM) and metabolic characteristic were evaluated on bacterial growth and their sulphur oxidation activities. The optimum pH of all the potential isolates occurred at pH 8.0. Meanwhile, the optimum temperature for isolate AH18, AH25 and AH28 occurred at 45 °C, 30 °C, and 30-45 °C, respectively. The three isolates were classified as facultative chemolithotroph with the capability of growth in thiosulphate concentration as high as 100 mM. Therefore, given the ability in the oxidation of thiosulphate, temperature and pH adaptabilities, with the metabolic flexibilities of isolates AH18, AH25, and AH28 could be a good H_2S biological deodorizing candidate.

Keywords: sulphur oxidising bacteria, hot spring, biological deodorisation, chicken manure, hydrogen sulphide

ABSTRAK

Dalam kajian ini, proses isolasi bakteri pengoksidasi sulfur (SOB) dari sumber mata air panas di Malaysia dilakukan dalam media kultur dengan menggunakan natrium tiosulfat sebagai satu-satunya sumber energi dan CO_2 sebagai sumber karbon tunggal. Sebanyak 80 isolat SOB diperoleh dari media agar dan dianggap sebagai SOB positif karena kemampuannya menggunakan tiosulfat untuk pertumbuhan. Semua isolat pada awalnya ditapis berdasarkan pada pertumbuhan yang cepat dalam media pertumbuhan cair dan 13 isolat telah terpilih untuk proses penapisan berikutnya. Tiga isolat SOB, yaitu isolat AH18, AH25, dan AH28 dipilih berdasarkan kemampuan pertumbuhan yang cepat, menghasilkan ion sulfat tertinggi, dan menurunkan pH dalam medium pertumbuhan. Berdasarkan pengamatan mikroskopis, semua sel bakteri yang diuji merupakan bakteri Gram-negatif dan berbentuk batang pendek. Berbagai peubah diuji termasuk variasi berbagai suhu (25-45 °C), pH (4-9), konsentrasi natrium tiosulfat (4-100 mM), dan karakteristik metabolik diamati pada kemampuan pertumbuhan bakteri dan aktivitas oksidasinya. Nilai pH optimum bagi semua isolat pontensial adalah 8.0. Sementara itu, suhu optimum untuk isolat AH18, AH25, dan AH28 masing-masing adalah 45 °C, 30 °C, dan 30-45 °C. Ketiga isolat tersebut diklasifikasikan sebagai *chemolithotroph* fakultatif dengan kemampuan pertumbuhan pada konsentrasi tiosulfat setinggi 100 mM. Oleh karena itu, berdasarkan kemampuan bakteri mengoksidasi tiosulfat, pertumbuhan pada berbagai suhu dan pH, dengan fleksibilitas metabolik isolat AH18, AH25 dan AH28 bisa menjadi penghilang bau H_2S secara biologis yang efisien.

Kata kunci: bakteri pengoksidasi sulfur, mata air panas, penghilang bau H_2S secara biologis, ekskreta, hidrogen sulfida

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INTRODUCTION

Poultry farm emits a large number of odorous gases such as ammonia, carbon monoxide, carbon dioxide, methane, hydrogen sulphide, dimethylamine, mercaptans, and phenolic compounds (Gutarowska *et al.,* 2014) which derived from the manure. However, among all of the manure gases, hydrogen sulphide (H_2S) is known as the most toxic and dangerous gas. It can be identified by its characteristic smell of rotten eggs which causing community problems by creating an unpleasant condition of working and living environment. In addition, H_2S is generated in lower abundance but significantly contributes to the total odorous nuisance due to their very low odour threshold limits (Kim *et al.,* 2007). This situation has created never-ending conflicts between the poultry farm and the surrounding residential area. Therefore, removal or reduction of H_2S, especially in the chicken manure, is necessary.

The removal of H_2S can be done through physicochemical methods including by the effective use of oxidising biocides such as chlorine (Cl_2), hydrogen peroxide (H_2O_2), and sodium hypochlorite (NaClO) (Oprime *et al.,* 2001). However, the processes based on such agents are expensive due to the high cost involved in the facility installation, as well as the operational cost and the use of toxic chemical may have a greater tendency to generate secondary pollution (Dehghanzadeh *et al.,* 2011). On the other hand, the biological method by using microorganisms has drawn much attention due to its cost-effectiveness with higher removal efficiency and environmentally friendly (De Gusseme *et al.,* 2009; Lin *et al.,* 2013). Biological treatment works on the principle which microorganisms such as bacteria act as a catalyst for the conversion of volatile pollutants into a less harmful form.

Sulphur oxidising bacteria (SOB) play a key role in the removal process of H_2S and other reduced inorganic sulphur compounds (thiosulphate, sulphite, and elemental sulphur) due to the fact that these compounds can serve as an energy source and/or a carbon sources for bacterial metabolism and thus, the harmful gasses can be eliminated. SOB is physiologically diverse, which can be characterised by a wide variety genera, broad habitat diversity, and can be classified by their metabolic characteristics including heterotrophic, mixotrophic, chemolithotrophic (colourless bacteria), and photoautotrophic (green and purple bacteria) (Ehrlich & Newman, 2009). There is a variety of SOB that have been employed to remove H_2S biologically and the most widely study SOB reported belongs to *Thiobacillus* spp. including *Thiobacillus thioparus* (Chung *et al*., 2007) which classified as a chemolithotrophic SOB. On the other hand, chemoorganotrophic SOB such as *Pseudomonas* spp. and *Xanthomonas* spp. have also been reported to be capable of oxidising H_2S (Xu *et al.,* 2016; Ho *et al*., 2008; & Chung *et al.,* 1996).

Sulphur oxidising bacteria can be found in a variety of environments including soil, water, and geothermal area. They can be found in the environment where the sulphur compounds are in higher concentrations which can provide good habitats for SOB (Druschel *et al.,* 2003). Hot spring is believed to have a high amount of saturated sulphur and various reduced sulphur compounds in the water due to the mud or the sediment are composed of sulphur compounds, which can potentially act as electron donors for SOB growth (Watsuntorn *et al.,* 2017). Additionally, microorganisms that live in hot spring water have minimal requirements for nutrients and their metabolic activities only depend on the biogeochemical cycle such as sulphur and other mineral contents (Skirnisdottir *et al.,* 2000). It is therefore of importance to isolate and select a potential SOB that can oxidize reduced inorganic sulphur compounds including H_2S, sulphide, sulphur, thiosulphate, and grow well at variable temperatures and pH. To our knowledge, the study on isolation of SOB inhabit hot spring water in order to reduce the H_2S in poultry manure is not available publicly. Thus, the objective of this study is to isolate and characterize the potential SOB from hot spring water in Malaysia and to evaluate its potential application in biological deodorisation of H_2S in poultry manure.

MATERIALS AND METHODS

Sample Collection

The hot spring water was collected from three different hot springs located in Malaysia which is Poring hot spring Sabah (6°02′44.9″N 116°42′13.5″E), Selayang hot spring (3°15′32.2″N 101°38′47.6″E), and Sungai Serai hot spring Selangor (3°05′28.0″N 101°47′42.0″E). The samples sources were collected in 1 L sterile Schott bottle at a depth of 0.5 m from the water surface and transported to the research laboratory. The temperature and pH of the hot spring water were measured *in situ*. The samples were stored at 4 °C until further analysis.

Medium and Culture Conditions

The thiosulphate mineral medium (TSM) composition is as followed (g/L): 1.5 g K_2HPO_4, 1.5 g KH_2PO_4, 0.4 g NH_4Cl, 0.8 g $MgCl_2.6H_2O$, 0.1 g $CaCl_2.2H_2O$, and 10 g $Na_2S_2O_3{\cdot}5H_2O$. The pH of the medium was adjusted with 1 M NaOH or HCl to 7.5 and solid media was prepared by addition of 15 g/L (1.5%) agar to the medium. The medium was sterilized in an autoclave at 121 °C for 20 min prior to its use.

Quantification of Sulphate Ion

The concentration of sulphate ion which was produced during the growth of SOB was determined by the method of Cha *et al.* (1999). Barium chloride ($BaCl_2$) solution (10% w/v) was added to culture supernatant in 1:1 and mixed up vigorously until a white turbid solution of barium sulphate ($BaSO_4$) was produced. Afterwards, the $BaSO_4$ solution was measured at 480 nm by a spectrophotometer. The values obtained were compared with the sulphate standard curve (Kolmert *et al.,* 2000). The standard sulphate solution was made by dissolving potassium sulphate (K_2SO_4) in deionized water to a known concentration from 0 to 3 mM and then was added with a $BaCl_2$ solution which formed a white

turbidity as the chemical reaction. The sulphate ion concentration is directly proportional to the turbidity of the solution.

Enrichment and Isolation of Sulphur Oxidising Bacteria

The enrichment was performed by placing 1 mL of liquid sample in a 250 mL Erlenmeyer flask containing 50 mL of sterilized TSM media with an initial 40 mM concentration of thiosulphate and incubated at room temperature (28 ± 2°C) with 160 rpm of agitation speed for 14 d until the medium turned turbid. After the incubation period, about 0.1 mL of the cultures were diluted in series (10^{-2} to 10^{-4}) and then spread evenly onto TSM agar medium plate. The inoculated agar plate was incubated at room temperature (28 ± 2°C) for 72h until the bacterial colonies were observed. The well-defined colonies were picked and re-streaked in fresh TSM agar to obtain pure cultures. A total number of 80 colonies were selected based on different colonial morphologies. The pure isolates obtained were labelled according to their sampling area and ecologies. The pure isolates were then sub-cultured by transfering of a single colony to fresh TSM growth medium broth and incubated at room temperature (28 ± 2°C) with 160 rpm of agitation speed for 14 d. Among these cultures, 13 isolates were selected based on their abilities to grow faster in the growth medium.

Screening of Potential Sulphur Oxidising Bacteria

The screening process was carried out using TSM broth with the addition of 0.01 g of bromocresol purple as the colour indicator of the pH changes. About 10% (v/v) of bacterial inoculum was inoculated and incubated at room temperature (28 ± 2°C) aerobically with 160 rpm agitation speed for 14 d. The procedure was carried out in triplicates and the potential SOB isolates were screened on the basis of their efficiencies in thiosulphate oxidation by producing the highest sulphate ion concentration and lowering pH in the growth medium (Ullah *et al.*, 2013).

Phenotypic Characterisation

Cell morphology of the isolate AH18 was observed under a light microscope (NOVEX B-range) at 1000x magnification with oil immersion. Gram staining was performed by the Hucker method. The presence of catalase activity was determined by the formation of bubbles with 3% (v/v) hydrogen peroxide solution and oxidase activity was determined with an oxidase test strip (Oxoid™). The biochemical characteristics of the isolates on organic carbon substrate utilization were determined by using an API20E kit (BioMérieuxs, Marcy I'Etoile, France) and carried out according to the manufacturer's instructions.

Effects of Physicochemical Properties on Microbial Growth and Sulphate Production of the Potential SOB Isolate

TSM medium containing thiosulphate (40 mM) as the electron donor under oxic conditions was used to determine the bacterial growth and sulphate ion production of the following changes to growth conditions:

1. pH of 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. The cultures were incubated at room temperature (28±2°C) with 160 rpm agitation speed for 7 d.
2. Temperatures of 25, 30, 35, and 45°C. The cultures medium were set at pH 8.0 and incubated with 160 rpm agitation speed for 7 d.
3. Various thiosulphate concentrations were tested. 5 mM and 10 mM was set as minimum concentration (Olguin-Lora *et al.*, 2011), 40 mM as initial concentration and 100 mM as a maximum concentration (Makzum *et al.*, 2016). The cultures media were set at pH 8.0 (optimum pH) and incubated at a temperature of 30 °C (optimum temperature) with 160 rpm agitation speed for 7 d.

For bacterial growth, 1 mL samples were withdrawn every 24 h and optical density was measured by spectrophotometer at 660 nm. The specific growth rate during logarithmic growth was determined for each temperature, pH, and various thiosulphate concentration. For final sulphate ion concentration, about 2 mL samples were withdrawn after 7 d of incubation and the concentration of sulphate ion was determined by using $BaCl_2$ as described by Cha *et al.* (1999) using spectrophotometer at 480 nm. The procedure was carried out in triplicates.

Metabolic Characterisation of the Potential SOB Isolate

The ability of the potential SOB isolates to grow chemolithotrophically on different reduced inorganic sulphur compounds was tested in the TSM medium supplemented with one of the following compounds; sodium sulphide (5 mM), tetrathionate (10 mM), thiocyanate (5 mM), and elemental sulphur (0.1% w/v). Chemoorganotroph growth was determined by using the TSM medium in which the sodium thiosulphate was replaced with the following organic compounds added at a concentration of 0.1% (w/v) for organic nitrogen and 0.2% (w/v) for carbohydrates (Vlasceanu *et al.*, 1997) and incubated at 30 °C aerobically for 3 d. The following organic compounds tested were yeast extract, peptone, glucose, and sucrose. Meanwhile, for the mixotrophic growth test was determined in the TSM medium which was amended with 0.05% (w/v) of yeast extract (Chen *et al.*, 2004) and incubated at 30°C (optimum temperature) aerobically for 7 d.

For the mixotrophic growth, about 1 mL of broth was periodically sampled every 24 h to measure the optical density of cell growth with a spectrophotometer at 660 nm. The specific growth rate of the isolate was determined and sulphate ion production was measured by using $BaCl_2$ as described by Cha *et al.* (1999). Moreover, inoculated TSM medium without yeast extract was used

as a control for the cell growth and sulphate ion production comparison. For chemolithotrophic growth, the sulphate ion production was measured and turbidity was observed and compared with control (without SOB). Meanwhile, for the chemoorganotrophic growth, the turbidity of broth was compared to the control (without SOB). The procedures were carried out in triplicates for all the isolates in growth condition tested.

Statistical Analysis

All data were analysed using one-way ANOVA of Statistical Analysis System Package (SAS) Version 9.4 software. Duncan's Multiple Range Test was performed to determine differences in the mean of treatments. P value was set at 0.05.

RESULTS

Isolation and Screening of the Potential SOB Isolate

The approach of the current study was to isolate and screen a potential SOB from hot spring water in Malaysia. The samples were collected from three different hot springs in Malaysia, the pH and temperature were measured *in situ* (45-55°C, pH 7.5-7.8). After the enrichment and purification processes, a total number of 80 isolates were obtained and the colonies were choosing based on the size, colour, and shape. At this point, the colonies obtained from the isolation were considered as a positive SOB due to their abilities using thiosulphate for growth. The 80 isolates were subjected to undergo a screening process in order to choose the potential SOB and thirteen isolates were found to have the capability to grow faster based on the development of turbidity observed. The thirteen isolates were undergone another screening process and based on the results obtained (Figure 1), three isolates were found to show a significant sulphate ion concentration ($P<0.05$) and reducing the pH of the medium (based on colour changes). Moreover, the three potential SOB has recorded a final pH in the range of 5.8–6.0 from the initial pH 7.5 in the broth using a pH meter. The three selected potential SOB was coded as isolate AH18, AH25, and AH28.

Phenotypic and Biochemical Characteristics of the Potential SOB Isolate

A single colony was observed appeared in less than 72 h incubation at room temperature (28 ± 2°C) on TSM agar. Isolate AH25 and AH28 had formed a regular white colony, smooth and creamy morphology whereas for isolate AH18 had formed white, a wrinkled colony with a star shape. Light microscopy examination of all isolates showed that cells were Gram-negative with short rods shape and the cell exhibited positive results for catalase and oxidase. By using API 20E, isolate AH25 and AH28 exhibited a similar biochemical characteristics behaviour, in which Arginine DiHydrolyse, Lysine DeCarboxylase, Ornithine DeCarboxylase, Urease, Citrate utilisation and Voges Proskauer were found to be positive whereas ONPG, Tryptophane DeAminase, Gelatinase, H_2S production, indole production and all the fermentation of carbohydrates were found to be negative. On the other hand, isolate AH18 exhibited different biochemical behaviours whereas the entire test was found to be negative except for Tryptophane Deaminase and fermentation of D-glucose and D-melibiose which showed positive results. Their phenotypic and biochemical characteristics are summarized in Table 1.

Effects of pH on Microbial Growth and Sulphur Oxidation Activity

To determine the effect of pH on SOB growth rate and sulphur oxidation activity, TSM medium was pre-

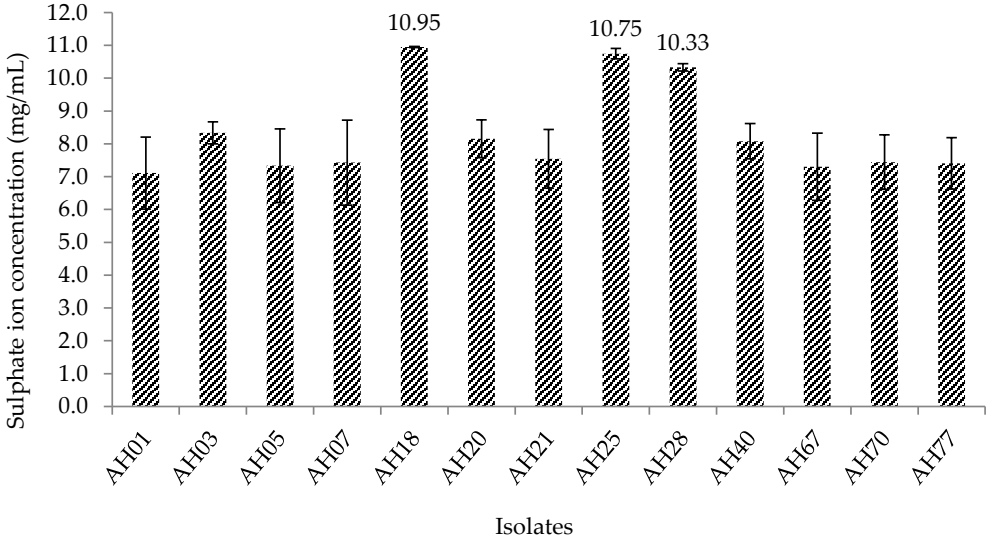

Figure 1. Screening results of sulphur-oxidising bacteria for sulphate ion production in TSM medium. Each point represents the mean of triplicate ±SE

pared with six different pH (4.0, 5.0, 6.0, 7.0, 8.0, and 9.0). As shown in Figure 2, the three potential SOB isolates were preferred to grow in the range of pH 6.0 to 9.0 accompanied by the production of sulphate ion in the growth medium. Based on the results, it was observed that the optimal pH of growth and sulphur oxidation activity was 8.0. However, the three isolates showed no growth activity and sulphate ion production in the pH medium of 4.0 and 5.0 (P>0.05).

Table 1. Phenotypic and biochemical characteristics of the potential SOB isolates

TRAITS	AH18	AH25	AH28
Phenotypic characteristic	Gram-negative	Gram-negative	Gram-negative
Cell type			
Motility	Motile	Motile	Motile
Shape	Rod	Rod	Rod
Oxidase	+	+	+
Catalase	+	+	+
API20E kit			
Enzymatic reactions			
ONPG	-	-	-
Arginine Dehydrolase	-	+	+
Lysine Decarboxylase	-	+	+
Ornithine Decarboxylase	-	+	+
Urease	-	+	+
Tryptophane Deaminase	+	-	-
Gelatinase	-	-	-
Citrate Utilisation	-	+	+
H2S Production	-	-	-
Indole Production	-	-	-
Voges Proskauer	-	+	+
Fermentation of carbohydrates			
Glucose	+	-	-
Mannitol	-	-	-
Inositol	-	-	-
Sorbitol	-	-	-
Rhamnose	-	-	-
Saccharose	-	-	-
Melibiose	+	-	-
Amygdalin	-	-	-
Arabinose	-	-	-

Note: (+) Indicate a positive test result, (-) Indicate a Negative test result

Effects of Temperature on Microbial Growth and Sulphur Oxidation Activity

The effects of temperature on the growth rate and sulphur oxidation activity of isolate AH18 are shown in Figure 3. The results showed that the three potential SOB isolates were able to grow over a broad range temperature investigated. Interestingly the microbial growth and sulphur oxidation activity of isolate AH18 and AH28 were observed increase with temperature increment and the optimal temperature was at 45°C (P<0.05) and 30°C to 45°C (P>0.05) respectively. Meanwhile, isolate AH25 shows a different bacteria growth and sulphur oxidation behaviour towards the temperature tested. The highest bacteria growth and sulphate ion production occurred at temperature 30°C (P<0.05) and was observed starts to decrease beyond that temperature.

Effects of Various Thiosulphate Concentrations on Microbial Growth and Sulphur Oxidation Activity

The concentration of thiosulphate tested in TSM was varied in a range of 5 mM to 100 mM. As shown in Figure 4, the microbial growth rate of isolate AH18 and AH25 was significantly increased with the increment of thiosulphate concentrations tested. Moreover, the increased in cell growth was also observed accompanied by the increase in sulphate ion production in which sulphate ion concentration on medium containing 40 mM and 100 mM thiosulphate showed the highest (P<0.05) sulphate produced compared to medium 5 mM and 10 mM. Nevertheless, isolate AH28 show a similar pattern in terms of sulphate ion production in which sulphate ion concentration was increased with the increment of thiosulphate concentration (P<0.05). However, there is no significant difference in term of cell growth observed for isolate AH28 for all the temperatures tested.

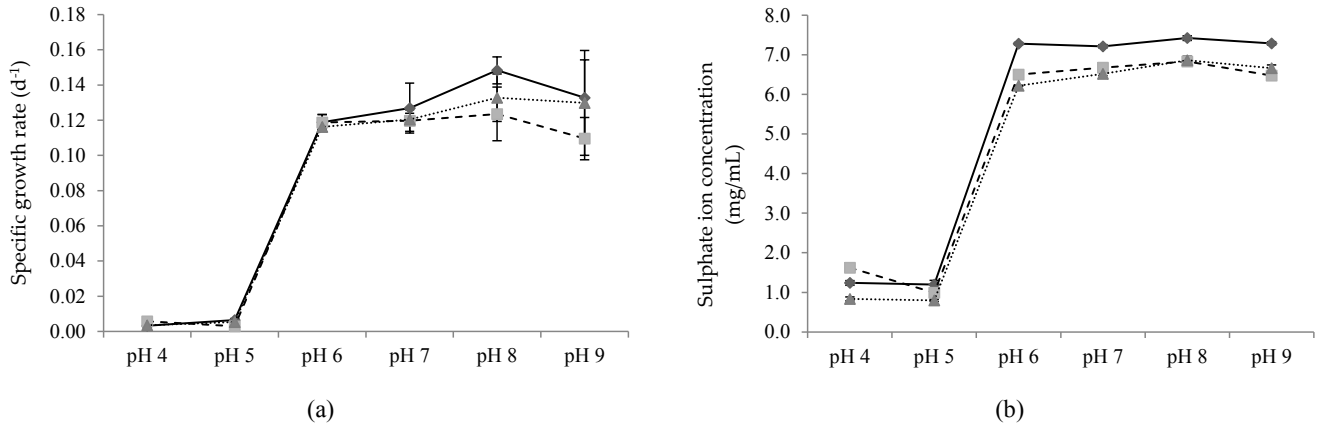

Figure 2. Bacterial growth rate (a) and sulphur oxidation activity (b) by isolate AH18 (-•-), AH25 (-■-), and AH28 (-▲-) at various pH. Each point represents the mean of triplicate ±SE.

Metabolic Characteristics of the Potential SOB Isolate

The chemolithotrophic growth of the isolate AH18, AH25 and AH28 occurred on growth medium supplemented with sulphide, tetrathionate, and elemental sulphur but not on thiocyanate. The ability of isolates to grow on the supplemented substrates was based on the turbidity observed and sulphate ion concentration mea-

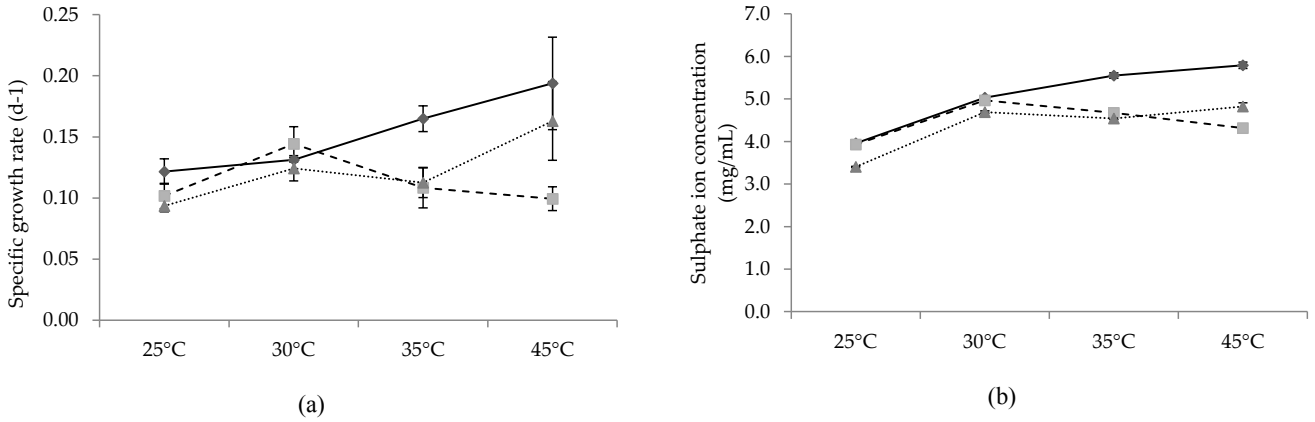

Figure 3. Bacterial growth rate (a) and sulphur oxidation activity (b) by isolate AH18 (-•-), AH25 (-■-) , and AH28 (-▲-) at various temperatures. Each point represents the mean of triplicate ±SE.

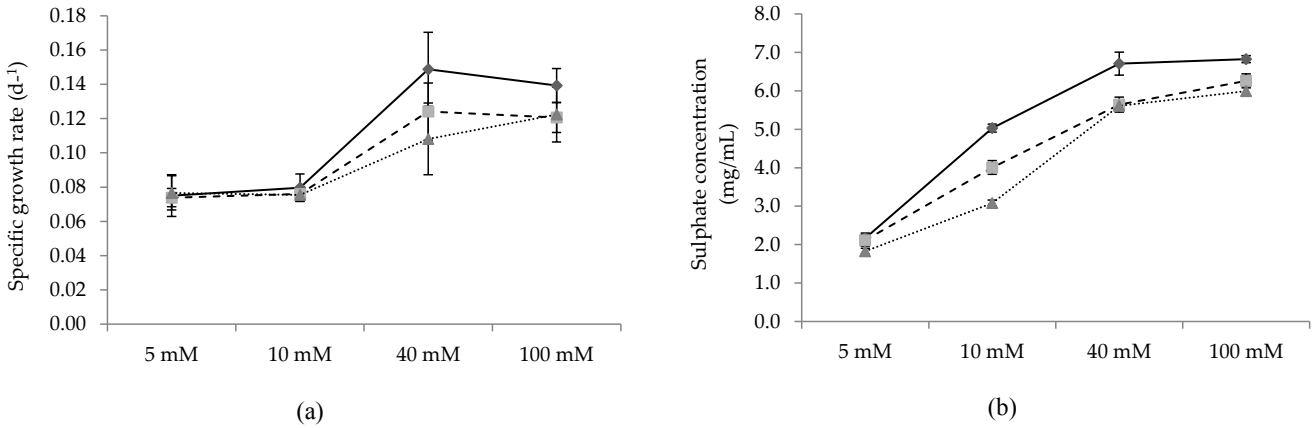

Figure 4. Bacterial growth rate (a) and sulphur oxidation activity (b) by isolate AH18 (-•-), AH25 (-■-), and AH28 (-▲-) at various thiosulphate concentrations. Each point represents the mean of triplicate ±SE.

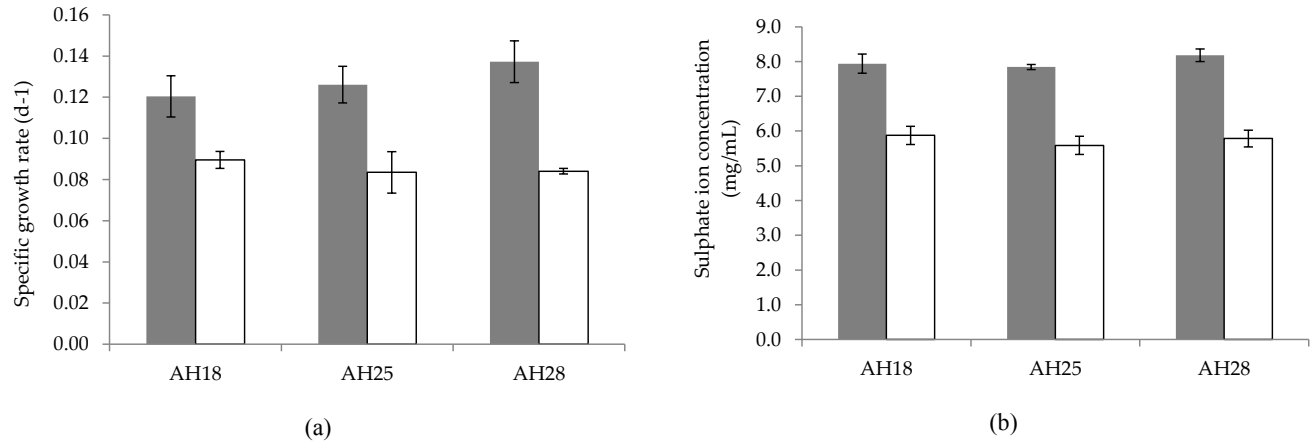

Figure 5. The specific growth rate (a) and sulphate ion production (b) of isolate AH18, AH25, and AH28 on TSM medium amended with 0.05% (w/v) of yeast extract (■) and without yeast extract (□). Each point represents the mean of triplicate ±SE.

surement. Meanwhile, chemoorganotroph growth of isolate AH18 on medium supplemented with peptone, yeast extract, glucose, and sucrose without any reduced inorganic sulphur compound showed a positive growth. On the other hand, isolate AH25 and AH28 showed negative growths in medium supplemented with peptone, glucose and sucrose except in yeast extract. Furthermore, the observation was similar to the result obtained in carbohydrate substrate tested with API 20E which showed negative results for all carbohydrate substrate tested on isolates AH25 and AH28. Interestingly, all the isolates were observed to grow rapidly when the TSM medium was amended with yeast extract for mixotrophic growth. As shown in Figure 5, the microbial growth rate and the production of sulphate ion were significantly higher ($P<0.05$) in medium amended with yeast extract compared to the medium without yeast extract for all the isolates.

DISCUSSION

The present study dealt with the isolation, screening, and characterisation of SOB from hot spring water in Malaysia. Three out of 13 isolates were selected as potential sulphur oxidizer bacteria for their ability to oxidize thiosulphate efficiently and produce the highest sulphate ion. In this study, thiosulphate was used in the enrichment and isolation medium as a selective substrate without the addition of any particular carbon sources order to stimulate the consumption of thiosulphate by the bacteria as a sole energy source. Additionally, thiosulphate has been used as the substrate for isolation of SOB because it is more readily soluble in water and non-toxic at a high concentration (Kuenen *et al*. 1992). The potential SOB isolates were also able to reduce pH in the growth medium which was a consequent of sulphuric acid production resulting from the biological oxidation of thiosulphate that was also reported by Hassan *et al.* (2010) and Yang *et al.* (2010) in their studies. However, in this study, the formation of sulphuric acid has reached a level in which is not favourable for SOB and brings the growth to a halt state. Similar to the works of Watsuntorn *et al.* (2017), which have observed no bacteria growth at a pH below 7.0 of SOB strain MAL 1HM19 isolated from hot spring in Thailand. Moreover, *Hydrogenobater* sp. which was also isolated from hot spring by Skirnisdottir *et al.* (2001b) could only reduce the thiosulphate medium to pH 5–5.5 from the initial pH of 7.3. Similar to the study of Vidyalakshmi & Sridar (2007) in isolation of *Thiobacillus* from various sources, 14 out of the 28 isolates were screened based on their efficacies to reduce the pH of growth medium from 8.0 to 5.0.

Most of the microorganisms prefer a specific pH range in which a change in pH can affect their growths and activities. Thus, it is important to investigate the optimal pH range of the microorganisms. In this study, the microbial growth and sulphur oxidation activity of all the potential SOB isolates were observed occurred under the neutral and slightly alkaline conditions with the optimum pH was 8.0. From the literature, the optimum pH for SOB was varied depending on the microbial habitat. The *Thioalkalivibrio jannaschii* isolated from hypersaline alkaline Mono Lake grow optimally at pH 10 (Sorokin *et al.,* 2002), while *Thiobacillus thiooxidans* isolated from highly acidic Crater Lake is able to grow down to pH 2.0–3.5 (Takano *et al.,* 1997). This result was strengthened by the fact that, hot spring water in Malaysia is known as the non-volcanic origin and most of the pH ranging between the values of 7.0 to 9.0 (Baioumy *et al.,* 2015). Thus, the pH preference of the isolate in this study is not surprising since the enrichment condition used is in a neutral condition (pH 7.5) as well as the environment pH from which it is isolated. However, the growth of all the potential isolates was inhibited under acidic condition. This could be due to the deterioration of the microbial growth activity and the metabolism activity under the inappropriate pH conditions and thus indicated the neutrophilic character of all the potential isolates. Moreover, the inhibition of growth under acidic condition was also supported by the small pH reduction during the screening process. The optimum pH obtained in this study is within the range reported for other SOB (Vikromvarasiri *et al.,* 2015; Park *et al.,* 2011). In addition, several representatives of *Thiobacillus* sp. have shown neutrophilic character such as *Thiobacillus novellus* and *Thiobacillus thioparus* with optimal pH of 7.0 and 7.5, respectively (Pokorna & Zabranska, 2015). Therefore, from the practical point of view, the ability of potential SOB to grow on varies pH conditions (except on acidic condition) would help the isolates to cope with the variable pH in biological deodorisation process since the pH of chicken manure is under neutral to alkaline condition.

The temperature requirements of some SOB are different and mostly they were found as mesophilic or thermophilic bacteria. In this study, all the potential SOB were capable of growing in the range of temperature tested (25-45 °C) with the different optimum temperatures recorded. However, the activity of isolate AH25 was observed decreasing with the increment of temperatures tested and the cell growth was observed slower at a temperature of 45 °C compared to the other isolates. This is understandable that the SOB inhabits the hot spring has undergone a physiological adaptation during the storage time as well as during the enrichment process at room temperature and thus isolate AH25 was classified as a mesophilic SOB. This was in line with Watsuntorn *et al.* (2017) study, in which only recorded an optimal temperature at 35 °C in SOB of strain MAL 1HM19 which was isolated from hot spring with *in situ* temperature at 70 °C. In fact, the ability of the potential SOB isolates to grow up to 45 °C was due to the natural habitat of bacteria itself which is from the hot spring. Meanwhile, isolate AH18 and AH28 have recorded an optimal temperature up to 45 °C and can be classified as moderate thermophilic bacteria which would be an advantage for these isolates for other use. Skirnisdottir *et al.* (2001a) isolated *Thermus scotoductus* from hot spring in Iceland which capable of growing and consuming thiosulphate at a high temperature of 65 °C. Hence, well adapted to variables temperature could be advantages for the isolate for *in situ* biological deodorisations in the tropical region.

Studies have shown that all the potential isolates were able to grow in thiosulphate concentration up to 100 mM in which, the microbial growth of the isolates was influenced by the amount of thiosulphate concentration added in the growth medium. This observation was in line with the study of Spring *et al.* (2001) which observed the increase in cell growth was accompanied by the increment of thiosulphate concentration in the medium of *Limnobacter thiooxidans*. This result can be explained by the availability of the substrate (thiosulphate) which was enough to supply energy to the bacteria for growth and produced significant sulphate ion concentration. In contrast with Skirnisdottir *et al.*, (2001b) works, which the increases in thiosulphate concentration has affected the conversion of thiosulphate to sulphate by *Hydrogenobacter* sp. and this could be explained due to the thiosulphate toxicity at high concentration which repressed the thiosulphate oxidation ability by the bacteria (Lee *et al.,* 2000).

All the potential isolates were classified as a facultative chemolithotroph because exhibited both chemolithotroph and chemoorganotroph (heterotroph) growth. It can be seen by the turbidity observed and the presence of sulphate ion in the culture medium which indicated that the isolates were able to oxidise the supplemented reduced inorganic sulphur compounds as an energy source for growth. However, no bacterial growth was observed in the medium supplemented with thiocyanate as an energy source as the bacteria grew extremely poorly under this condition. Similarly, Shi *et al.* 2011 and Chen *et al.* 2004 also have reported that SOB isolates unable to grow on medium supplemented with thiocyanate as an energy source. This could be due to the lack of enzyme that is used as a catalyst to break down the thiocyanate molecule. From the literature, thiocyanate is a chemically stable compound and only a few chemolithotrophic SOB are capable of using thiocyanate as an electron donor for growth. In Bezsudnova *et al.* (2007) study, a novel halophilic SOB *Thiohalophilus thiocyanoxidans* was capable of growing with thiocyanate as an electron donor for growth and have recorded a high cyanase activity. Similar to Sorokin *et al.,* (2014) which has proven the obligately chemolithoautotrophic *Halothiobacillus* strain was capable of using thiocyanate as an energy source for growth. Interestingly, all the isolates were observed prefer to chemoorganotroph grow in medium supplemented with yeast extract compared to other organic compounds. This can be validated by the results of biochemical characteristics (Table 1) in which the isolates AH25 and AH28 were unable to utilise all the carbohydrate substrates tested. Additionally, based on the biochemical test results, isolates AH25 and AH28 could be identical species because of exhibiting similar biochemical behaviour whilst isolate AH18 was slightly different. This observation was similar to Kantachote *et al.* (2008) works in which using API 20E kit to validate the utilisation of organic compounds. Moreover, yeast extract has become favourable organic compound by all the potential SOB due to various of amino acid and peptides contents in yeast extract and the water-soluble characteristic of vitamins and carbohydrates molecule which are enough to support the bacterial growth of the isolates. However, by adding organic compound (yeast extract) into the corresponding TSM liquid medium, the bacterial growth was significantly enhanced. In addition, the greatest sulphate ion formation also was observed in medium containing both thiosulphate and yeast extract compared with medium containing the only thiosulphate. Thus, it appears that the isolates preferred mixotrophic growth because the bacteria grow better with the presence of both reduced inorganic sulphur compound and an organic compound. Similar works have also reported that mixotrophy growth with yeast extract was found to be a great condition which promotes the growth of bacteria and the oxidation process of sulphur compounds resulting in higher sulphate ion production (Vardanyan & Vardanyan, 2014; Kantachote *et al.,* 2008; Chen *et al.,* 2004). Hence, the metabolic flexibility might ensure better survival and the growth of SOB in various environments (Graff & Stubner, 2003), especially in the absence of reduced inorganic sulphur compounds sources.

It is important to determine the adaptability and feasibility of the SOB in the various environmental conditions as described above to ensure their effectiveness in biological deodorisation performance. Therefore, it was ascertained from this present investigation that the potential SOB isolates have possessed a good application potential for the biological deodorisation of H2S in chicken manure due to their ability to perform the oxidisation of sulphur compound at various parameters tested.

CONCLUSION

It was concluded that three potential SOB isolates were successfully isolated from hot spring in Malaysia which have remarkable potentials for application in the biological deodorisation of H_2S in chicken manure. The results of the present study revealed that the potential isolates of AH18, AH25, and AH28 capable of oxidising of thiosulphate up to a concentration of 100 mM. The optimum pH was 8.0 and optimum temperatures of isolate AH18, AH25, AH28 were at 45 °C, 30–45 °C, and 30 °C, respectively, which makes them appropriate candidates for the biological deodorisation. Moreover, all the potential isolates were classified as facultative chemolithotroph and this metabolic versatility could be an advantage to survive in various environmental conditions. All the data gathered in this study could be useful information in developing a feasible biological deodorisation strategy.

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